FINE STRUCTURE OF *BODO SALTANS* AND *BODO CAUDATUS* (ZOOMASTIGOPHORA : PROTOZOA) AND THEIR AFFINITIES WITH THE TRYPANOSOMATIDAE

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SYNOPSIS

Characters specific to each of two species of *Bodo* are described for the first time. *Bodo saltans* possesses cytoplasmic bacteria, hair-like appendages (mastigonemes) on the anterior flagellum and circumbuccal lappets surrounding the opening of the alimentary system. In *B. caudatus*, an electron dense band separates the kinetoplast from the basal bodies of the flagella. In addition, clear differences exist between the microtubular systems associated with the buccal cavity and cytopharynx. It is suggested that the mastigonemes and circumbuccal lappets of *B. saltans* are responsible for the capture of food organisms.

In both species the alimentary system is a membrane-lined tube surrounded by a number of microtubules. The single mitochondrion is dilated in the vicinity of the basal bodies and contains a prominent kinetoplast. Since both these organelle systems closely resemble those found in members of the related Trypanosomatidae, the possible origin of this family from *Bodo* or a *Bodo*-like flagellate is discussed.

INTRODUCTION

Bodo is a cosmopolitan flagellate found in fresh and brackish waters and in some soils. This and other members of the Bodonidae are of interest chiefly because of their close relationship to the economically and medically important trypanosomes.

This relationship is based on the presence of a mass of DNA—the kinetoplast—situated in a dilatation of the single mitochondrion. In trypanosomes, this organelle has been regarded as a genetic system containing the information required for the synthesis of mitochondrial enzymes (Steinert, 1960) but in *Bodo* its function has not been examined. Of the many species of *Bodo* which have been described, *Bodo saltans* Ehrenberg, 1831 and *Bodo caudatus* Stein, 1878 are probably the two most commonly found. They occur in quite different habitats for whereas *B. caudatus* tends to be coprozoic, *B. saltans* is usually found in freshwater. Separation of the two species depends on such characters as body shape and size, length of the flagella and the position of the nucleus relative to that of the kinetoplast. However, the present fine structural study describes a number of clearly defined qualitative differences between *B. saltans* and *B. caudatus* which would not have been visible to the earlier light microscopists.

MATERIALS AND METHODS

Bodo saltans was isolated from a sample of fresh water taken from a pond near Slapton Ley, Devon. From this isolate, clone cultures were established on 0·1% w/v 'Oxoid' dehydrated liver infusion (pH 4·6) and maintained at 25 °C.

Bodo caudatus was isolated from an infusion of pig faeces which was obtained from Winches Farm, near St. Albans, Hertfordshire. Clone cultures from this isolate were maintained at 25 °C on 0·2% w/v 'Oxoid' beef extract (pH 5·8). In both cases, cultures were agnotobiotic. For light and electron microscopy, cultures were harvested after 3 days growth.

Light microscopy.—Phase contrast observations were made using a Leitz Ortholux microscope fitted with a Heine condenser. Flagellates were examined either alive or after fixation with 1% osmium tetroxide. Smears fixed in Schaudinn's fluid were stained with iron haematoxylin and examined by bright field microscopy.

Electron microscopy.—Flagellates were collected by centrifugation at 1,000 r.p.m. for 10 minutes and the resulting pellet fixed for 5 minutes at room temperature in 1% osmium tetroxide buffered to pH 7·4 either with 0·1 M Sorensen's phosphate buffer or 0·1 M veronal acetate (Michaelis). Before dehydration, the pellet was treated with 1% uranyl acetate in 25% ethanol for 30 minutes. After dehydration in ethanol-water mixtures and absolute ethanol, the pellet was treated with propylene oxide or toluene and embedded in Araldite. Sections were cut using a Porter-Blum MT2 ultramicrotome and stained in lead citrate prior to examination in an EM 6B electron microscope.

Some flagellates were fixed as before, washed in distilled water and dried onto grids. They were then placed in a coating unit and shadowed with gold/palladium at an angle of 30°. Negatively stained preparations were made using sodium phosphotungstate at pH 7·0.

RESULTS

LIGHT MICROSCOPY.—*Bodo saltans*.—The body of this flagellate is oval in shape (5–8 μm long and 2–5 μm wide) and has two flagella of unequal length which arise from the bottom of a depression (the reservoir or flagellar pocket) near the anterior

end of the cell (Fig. 1). During locomotion in which the body rotates on its own axis, the shorter anterior flagellum is extremely active while the posterior flagellum remains stationary or undergoes slight movement. When stationary, the flagellate often attaches itself to some object by the tip of its trailing flagellum and may then exhibit rapid oscillations. This behaviour is characteristic of *B. saltans*. The kinetoplast lies near to the basal bodies of the flagella and the nucleus, which con-
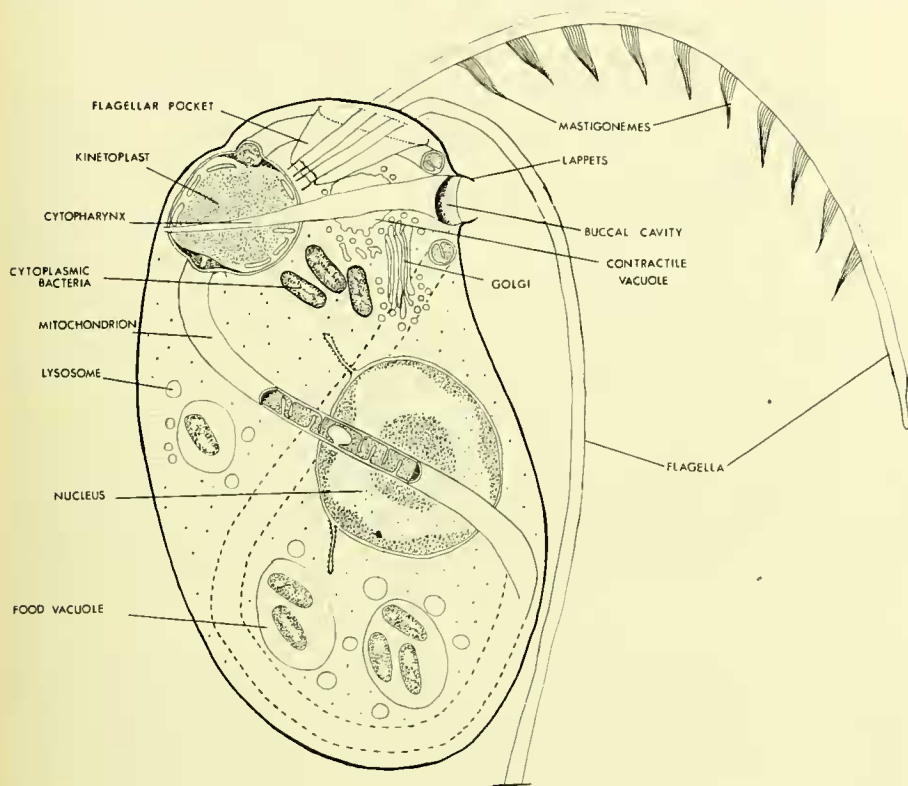

Fig. 1. Diagram of *Bodo saltans* showing the arrangement of the main organelles.

tains a conspicuous central karyosome, is central or mid-ventral in position. The single mitochondrion is markedly siderophilic and appears as a thin filament which originates from one side of the kinetoplast, describes a figure of eight or a loop within the cell and terminates at the opposite side of the kinetoplast. A single, round contractile vacuole appears at intervals at the anterior end of the living cell. It is situated just below and to one side of the flagellar pocket into which it periodically discharges its contents. The posterior half of the flagellate is occupied by a variable number of food vacuoles.

The opening of the alimentary system, the buccal cavity, is marked by a small vacuole on the ventral surface near the anterior end of the flagellate. Ingestion of bacteria is a very rapid process and can be observed satisfactorily only during the short periods of quiescence when it is attached to the substratum by its posterior flagellum. During ingestion, a bacterium is drawn into the buccal cavity and rapidly passes ventro-dorsally along a path which corresponds exactly with that of the cytopharynx (see later). A large vacuole then appears at a point which probably corresponds to the end of the cytopharynx and the bacterium passes into it. This food vacuole then slowly moves to the posterior end of the cell.

Bodo caudatus. When harvested in the logarithmic phase of growth, *B. caudatus* is long and narrow with a convex dorsal and concave ventral surface (8–14 μm long and 4–6 μm wide). The two flagella are of unequal length and arise from the bottom of the flagellar pocket near the anterior end of the cell. The anterior flagellum is the shorter but most active during locomotion. Situated in the anterior half of the cell, the nucleus frequently lies very close to the kinetoplast. As in *B. saltans*, the single mitochondrion is continuous at both its ends with the kinetoplast and describes a loop-like circuit of the cell. Almost the entire posterior half of the flagellate is occupied by food vacuoles and an active contractile vacuole lies close to the flagellar pocket. Although anteriorly a small buccal cavity is clearly visible, the ingestion of bacteria is very difficult to observe because of the relentless swimming habit of this flagellate.

ELECTRON MICROSCOPY.—*Flagellar Pocket.* In both species of *Bodo*, the flagellar pocket is a lateral depression at the anterior end of the body. Because the two flagella emerge from the cell at the bottom of this structure (Fig. 1, Pl. 1, Fig. A), Pitelka (1961) referred to it as the circumflagellar depression. The flagellar pocket is lined with a unit membrane continuous with that covering the rest of the cell and below this, there lie a number of pellicular microtubules. Further details of these are given below.

Basal bodies and flagella. The two basal bodies are embedded in the cytoplasm at the base of the flagellar pocket and are therefore antero-lateral in position. They are structurally differentiated into 2 regions, an intracytoplasmic proximal portion and an extracytoplasmic transition zone which connects the intracytoplasmic portion to the flagellar shaft and is bounded by the flagellar membrane (Pl. 1, Fig. A). The proximal end of the basal bodies lies very close to the surface of the kinetoplast capsule (Pl. 1, Fig. A). The gap between these two structures is somewhat variable in width but is generally in the order of 100 nm and direct contact has never been observed. Under some conditions of fixation, a reticulum of fine filaments (8 nm wide) fills each basal body.

The junction of the basal body transition zone with the flagellum is marked by two transverse basal plates which are thickened peripherally (Pl. 1, Fig. B). The two central tubules of the axoneme originate just above the distal basal plate and the paraxial rod—a structure which runs parallel to the axoneme for about three quarters of its length—arises from a lateral extension of the proximal basal plate. The basal plate extensions of each flagellum face one another and the overlying flagellar

membranes are joined by a wide but very thin extracellular striated band (Pl. 1, Figs B and C). Of the three closely spaced striations, only the central one (5 nm wide) is prominent. Those lying on either side of it are more diffuse and can be satisfactorily resolved only in longitudinal sections of the band.

The transition zone of the basal bodies consists of 9 peripheral doublets of tubules each of which is joined to the adjacent flagellar membrane by a narrow connective. The intracytoplasmic portion is composed of 9 tubule triplets and is open proximally. The proximal ends of the basal bodies are connected by three striated rootlets (Pl. 1, Fig. D). Two of these arise from a single triplet on the basal body of the posterior flagellum and diverge slightly as they approach and insert onto the other basal body. A third rootlet running approximately parallel to the other two also connects the basal bodies. All three rootlets have a major period of 50 nm. The prominent bands delimiting the major repeating unit are 25 nm wide and between these lies a narrower 5 nm wide band. There is in addition, a 'Y' shaped rootlet which arises from the basal body of the anterior flagellum and passes antero-laterally to insert onto a group of 2–3 short microtubules running parallel to the cytopharynx (Pl. 1, Fig. D).

Both flagella contain the familiar '9+2' arrangement of tubules and a paraxial rod (Pl. 1, Fig. E). After running parallel to the axoneme for about three-quarters of its length, the paraxial rod tapers before terminating. Details of its structure are difficult to resolve but it appears to have a lattice-like architecture. The anterior flagellum of *Bodo saltans*, unlike that of *B. caudatus*, bears mastigonemes. These hair-like appendages are arranged in bundles along one side of the flagellum and have been reported elsewhere (Brooker, 1965). Metal-shadowed preparations of *B. saltans* show that the posterior flagellum bears a number of parallel transverse striations whose separation is 14 nm (Pl. 1, Fig. F). They are found only after the flagellum has emerged from the flagellar pocket and extend distally for a distance of 3 μm. In *B. caudatus* similar striations first appear at a level corresponding to the junction of the basal body with the flagellum but because they extend distally only for a distance of about 1 μm, they are not visible in shadowed material. In sectioned material, the striations are seen as periodic thickenings (14 nm wide) of both leaflets of the flagellar membrane (Pl. 1, Fig. E, Pl. 2, Fig. A). Such thickenings occupy about 25% of the circumference of the flagellar membrane (Pl. 1, Fig. E). On passing distally, this percentage gradually decreases so that in shadowed preparations of *B. saltans*, the array of striations appears to taper to an end (Pl. 1, Fig. F).

Alimentary system. The ingestion of food particles by *Bodo* takes place by way of permanent oral structures—the buccal cavity and cytopharynx—which lie to the right of the flagellar pocket (Pl. 2, Fig. B). The position at which the buccal cavity opens on to the surface of the cell differs in the two species. In *B. saltans*, it opens antero-ventrally (Pl. 2, Fig. C) but in *B. caudatus* it is found at the extreme anterior end of the flagellate (Pl. 2, Fig. D). Very rare sections in which a bacterium is found in the buccal cavity (Pl. 1, Fig. A) suggest that this organelle is capable of considerable distension. Frequently, the membrane lining the buccal cavity has a pronounced cell coat (Pl. 2, Fig. C) which takes the form of bundles of fine filaments projecting perpendicularly from the membrane. A number of structures, referred

to here as circumbuccal lappets, surround the opening of the buccal cavity of
B. saltans (Pl. 2, Fig. B). They are flat, triangular projections (Pl. 3, Fig. A) which
are joined at their bases to form a ring. Each lappet is composed of many fine
filaments and arises from an intracellular band at the margin of the buccal cavity.
These structures appear to be totally absent from *B. caudatus*.

From the left side of the buccal cavity arises an elongate tube lined by a unit
membrane and surrounded by a number of microtubules (Pl. 2, Fig. B). In accord-
ance with the terminology used for the oral apparatus of ciliates, this organelle will
be referred to as the cytopharynx. In *Bodo saltans* the cytopharynx approaches
the kinetoplast, passes underneath and to one side of it and on reaching the dorsal
surface of the body, curves to one side (Pl. 3, Fig. C). In *B. caudatus*, it passes
posteriorly just below the cell membrane (Pl. 2, Fig. D). Although the cytopharynx
terminates just posterior to the kinetoplast, the microtubules associated with it
frequently reach the posterior margin of the nucleus before terminating. In both
species, the diameter of the lumen decreases distal to the buccal cavity. Numerous
vesicles of variable diameter are always found underneath and to the right side of
the cytopharynx in *B. saltans* (Pl. 2, Fig. B, Pl. 3, Fig. C). Although similar
vesicles are occasionally found in *B. caudatus*, they are generally smaller and less
numerous. Their origin is not clear, but many longitudinal sections of the cyto-
pharynx suggest that they arise as invaginations of the cytopharyngeal membrane.

Both light and electron microscope observations suggest that ingestion of food
particles takes place at the blind end of the cytopharynx. Since the diameter of the
cytopharynx is at all points along its length smaller than that of food organisms
found in the food vacuoles, ingestion must be accompanied by considerable dis-
tension of the cytopharynx. Ingestion results in the formation of a number of
food vacuoles which migrate to the posterior half of the cell. The food vacuoles are
bound by a single unit membrane and may contain one or more food organisms
(Pl. 5, Fig. D). When digestion is complete, only a diffuse mass of undigestible
material remains in the vacuole. Since such vacuoles do not accumulate in the
cytoplasm, it seems likely that undigestible material is voided by the coalescence of
the cell membrane with that of the food vacuole. However, this has never been
observed.

Microtubular systems. Microtubules of external diameter 20–25 nm are associated
with several organelles in the anterior half of the flagellate. Many, but not all, of
the tubules which are associated with the flagellar pocket, cytopharynx and body,
appear to arise from the proximal ends of the two basal bodies.

Bodo saltans. The microtubules which are to surround the cytopharynx pass
along the flagellar pocket in two groups. At the opening of the flagellar pocket,
three members of each group approach each other (Pl. 3, Fig. B) and continue as
microtubule doublets. They curve over the narrow bridge of cytoplasm separating
the flagellar pocket from the alimentary system and enter the walls of the buccal
cavity where they adopt a 'U' shaped configuration. From the left side of this
cavity, the microtubules follow the course of the cytopharynx and become arranged
around it in two groups. Associated with the floor and left side of the cytopharynx
is a group of 5 microtubules and with the roof a group of 3 (Pl. 3, Fig. D). The right

side of the organelle is free of tubules. The middle 3 tubules of the group of 5 are double and joined on their left side to the cytopharyngeal membrane by a short connective. Although 8 microtubules normally run parallel to the cytopharynx, 9 or 10 are sometimes found (Pl. 3, Fig. E). The microtubules of each group are connected by a number of fine filaments which occur at intervals (13 nm) along their length (Pl. 4, Fig. A). Beyond the blind end of the cytopharynx, the connectives of the microtubule doublets disappear but the arrangement of the two groups of tubules remains constant until they terminate near the dorsal surface of the flagellate.

Beneath the cell membrane at the anterior end of the cell lie a number of pellicular microtubules. Although some of these arise from the basal bodies of the flagella, many appear to have their origin near the opening of the flagellar pocket. From here, the microtubules spiral round the anterior end of the cell and on approaching the right side of the buccal cavity dip below the cell surface (Pl. 4, Fig. B). They then describe a semi-circular path as a curved band of 15–20 tubules (Pl. 4, Fig. C). Passing below the buccal cavity (Pl. 3, Fig. C) they move anteriorly and terminate in the cytoplasm between the cytopharynx and the flagellar pocket.

Bodo caudatus. The arrangement of microtubules associated with the buccal cavity resembles that described for *B. saltans.* The tubules which are to surround the alimentary system pass along the wall of the flagellar pocket and enter the buccal cavity. Here, transverse sections show that the tubules are arranged in a line along the left wall (Pl. 4, Fig. D, Pl. 5, Fig. A). As the tubules follow the cytopharynx from the left side of the buccal cavity, they distribute themselves around the cytopharyngeal membrane (Pl. 5, Fig. B) in 2 overlapping groups. One group associated with the roof of the cytopharynx usually contains 4 microtubules; the other group, which lies next to the floor and left side of the organelle, may contain 4, 5 or 6 tubules three of which are double and joined as in *B. saltans* to the cytopharyngeal membrane by a short connective. Such connectives disappear a short distance from the buccal cavity.

Another set of microtubules emerges from the flagellar pocket, spirals round the anterior end of the cell and comes to occupy the right hand wall of the buccal cavity just below the lining membrane (Pl. 4, Fig. D). The 15–20 microtubules in this set are joined by intertubular connectives and for most of their length travel posteriorly parallel to the buccal cavity and cytopharynx. A limb of the single mitochondrion which passes beneath the buccal cavity modifies the path taken by some of the tubules. As they travel posteriorly, the tubules gradually move away from the cytopharynx (Pl. 5, Figs A, B) and towards the cell membrane until, at a level beyond the blind end of the cytopharynx, they are seen as a row of pellicular microtubules. These tubules travel some distance posteriorly before terminating. In addition to this major set, an equally conspicuous row of very short parallel tubules is found on either side of the buccal cavity (Pl. 4, Fig. D).

Kinetoplast-mitochondrion.—Lying just below the cell membrane, the single mitochondrion describes a loop-like circuit of the cell (Fig. 1). In *Bodo saltans*, this organelle travels above and parallel to the cytopharynx, describes a semi-circular path around the right side of the buccal cavity (Pl. 5, Fig. D) and passes to the extreme posterior end of the cell before returning anteriorly to the basal body region.

The whole circuit of the mitochondrion frequently takes the form of a figure of eight. The form of the mitochondrion in *B. caudatus* is similar to that of *B. saltans* except that in the posterior region of the former it is frequently seen as a branching structure.

The cristae are predominantly plate-like and arise from the inner mitochondrial membrane. The granular matrix of the mitochondrion is dense but sometimes contains irregularly shaped bodies of much greater electron density (Pl. 5, Fig. C).

In the region of the basal bodies, a prominent spherical dilatation of the mitochondrial tube houses the kinetoplast. This will be referred to as the kinetoplast capsule. The kinetoplast is composed of a complex reticulum of fine filaments ($2 \cdot 5$–$3 \cdot 0$ nm thick) and contains a large number of irregularly distributed electron dense nodes (Pl. 6, Fig. A). It is separated from the wall of the mitochondrion by a number of cristae embedded in a thick layer of mitochondrial matrix (Pl. 6, Fig. A). The basal bodies of the flagella lie very close to the surface of the mitochondrial kinetoplast capsule but are never seen in contact with it. In *Bodo caudatus*, the basal bodies are separated from the capsule by a flat electron dense pad (Pl. 1, Fig. A).

Nucleus.—The relative position of the nucleus within the cell is one of the features by which the two species of *Bodo* may be separated. In *B. saltans* it is found mid-ventrally some distance from the kinetoplast capsule whilst in *B. caudatus*, it is always found very close to this part of the mitochondrion. The nucleus is bound by a nuclear envelope composed of two membranes of which the outer is continuous with the granular endoplasmic reticulum. A prominent and finely granular nucleolus of variable shape occupies the centre of the nucleus (Pl. 5, Fig. D). In *B. saltans* a layer of condensed chromatin is often seen attached to the inner membrane of the nuclear envelope but in *B. caudatus*, this component of the nucleus appears to be absent or at least is not visualized by the techniques used in this study.

Endocytoplasmic bacteria.—Structures have been observed in the cytoplasm of *Bodo saltans* which, on morphological grounds, have been tentatively identified as bacilliform bacteria (approximately 1 μm long and $0 \cdot 3$ μm wide). They are found in all individuals of the flagellate population and are always situated in the anterior half of the cell (Pl. 2, Fig. C, Pl. 4, Fig. C). Although the largest number of bacteria seen in one cell profile is four, the total population is probably much larger. They can be easily distinguished from food organisms since the latter are separated from the cytoplasm of the flagellate by the membrane of the food vacuole and are usually found at the posterior end of the cell (Pl. 5, Fig. D).

Each bacterial cell is bounded by a cell membrane 8 nm thick which bears on its outer surface a thin layer of filamentous material in direct contact with the host cytoplasm. Extensions of a layer of dense material lying beneath the cell membrane project into the electron lucent central portion of the bacterium which is traversed by fine fibrils. Deep, mid-length constrictions of the bacteria suggestive of division are commonly encountered and appear independently of the host cell division cycle (Pl. 6, Fig. C).

Cytoplasmic membrane systems.—The contractile vacuole is situated on the left side of the flagellate just below and to one side of the flagellar pocket (Pl. 4, Fig. D). Surrounding the vacuole is a number of vesicles and tubules which serial sections

show to be continuous with the lumen of the vacuole (Pl. 6, Fig. B). After systole, the membrane lining the vacuole appears rounded in section. At discharge, the membranes between the flagellar pocket and contractile vacuole coalesce and the contents of the vacuole are discharged into the flagellar pocket. The thin layer of cytoplasm between the membranes of the flagellar pocket and contractile vacuole is traversed by a number of concentrically arranged circular septa. Coated vesicles are commonly encountered in the vicinity of the vacuole and often they are seen with their membrane confluent with that of the vacuole.

The Golgi apparatus is situated directly below the cytopharynx (Pl. 4, Fig. C, Pl. 5, Fig. D) and to the right side of the contractile vacuole and is composed of a stack of 6–10 compressed saccules. Although vesicles of both the smooth and coated type are actively proliferated from the margins of all the Golgi saccules, there is frequently a notable concentration in the region of the distal saccule.

Cisternae of granular endoplasmic reticulum arise from the outer membrane of the nuclear envelope and ramify throughout the cell. One limb of this reticulum is permanently associated with the posterior margin of the cytopharynx and runs parallel to it for most of its length (Pl. 3, Fig. D).

DISCUSSION

Since, at the level of the light microscope, *Bodo saltans* and *B. caudatus* appear to possess the same major organelle systems, separation of the two species is based on differences in the spatial arrangement of these organelles and on differences in the size and shape of the body. Whilst confirming the validity of such criteria, the present study has shown that separation is also possible using characters which are beyond the resolution of the light microscope. Thus in *B. saltans*, circumbuccal lappets, endocytoplasmic bacteria and mastigonemes on the anterior flagellum are consistently present but are never found in *B. caudatus*. Similarly, the dense layer of material which separates the basal bodies of the flagella from the kinetoplast capsule is present only in *B. caudatus*. Although Pitelka (1961) was only able to make a tentative identification of the flagellate she studied, it is clear that it was *B. saltans* for her pictures show the cytoplasmic bacteria and circumbuccal lappets of this species.

The alimentary system and the microtubules associated with it have been briefly reported by Pitelka (1961). She pointed out that the non-contractile rostral vacuole described by Hollande (1942) corresponded to the cup-like depression at the opening of the alimentary system, a structure which has been referred to as the buccal cavity in the present study. Sections of the alimentary system containing partially ingested bacteria suggest that the buccal cavity and cytopharynx are capable of considerable distension. This conclusion is supported by the observations made by Sinton (1912) on a flagellate which he referred to as *Prowazekia urinaria* but which, from his description, was probably *Bodo caudatus*. He observed that the flagellate was able to ingest not only large bacteria but also red blood cells and that on these occasions the buccal cavity was capable of being greatly distended. As Sinton describes it, the path taken by the bacteria through the cell during ingestion

corresponds exactly to the course of the cytopharynx described in the present study.
A similar conclusion has been drawn from the light microscope observations of
feeding in *B. saltans*.   These results are contrary to Pitelka's assertion that bacteria
do not pass along the cytopharynx.   Although the function of the microtubules
associated with the alimentary system is unknown, it is possible that they confer a
degree of elasticity on the organelle which enables it to return to its normal shape
and size once ingestion is complete.   Schuster (1968) suggested a similar function
for the cytopharyngeal tubules of the cryptomonad flagellate *Cyathomonas truncata*
and extended this proposal to the case of *Bodo*.

The capture of prey by Bodo *caudatus* has been described by Sinton (1912).
According to this author, the distal portion of the anterior flagellum is capable of
grasping bacteria and propelling them to the opening of the buccal cavity by coiling
movements.   Infolding movements of the edges of the buccal cavity then initiate
ingestion.   This mechanism is possible in *B. caudatus* only because the buccal cavity
opens anteriorly and is therefore ideally situated to receive bacteria carried to it by
the anterior flagellum.   Because the buccal cavity of *B. saltans* opens antero-
ventrally, the mechanism described above for *B. caudatus* does not appear adequate
to explain the capture of food organisms.   Instead, a mechanism involving the
mastigonemes of the anterior flagellum is proposed.   Although the mastigonemes
do not appear to play a major role in locomotion (Holwill, 1966), it is possible that
during the oar-like movements of the anterior flagellum (Holwill, 1966) they exert
a component force in the direction of the buccal cavity which sweeps food organisms
towards it.   Such a mechanism may bring bacteria to the vicity of the buccal
cavity but the initiation of ingestion probably depends on movements of the margins
of the buccal cavity as described by Sinton (1912) for *B. caudatus*.   In this process,
participation by the circumbuccal lappets may be important.   It is visualized
therefore that the mastigonemes and the circumbuccal lappets are functionally
integrated to form a system responsible for the capture and ingestion of food
organisms.   Schuster (1968) has described a filamentous fringe surrounding the
opening of the cytopharynx of *Cyathomonas* which, like the circumbuccal lappets of
B.saltans, has its origin beneath the cell membrane and is believed to assist in feeding.

In her study of *Bodo saltans*, Pitelka (1961) suggested that the cytopharynx was a
modified intracytoplasmic flagellum.   This suggestion was based on the observation
that the cytopharynx is surrounded by 9 microtubules and arises close to the surface
of the kinetoplast near the basal bodies of the flagella.   However, it has been shown
here that the cytopharynx passes beyond the kinetoplast capsule and although
occasionally surrounded by 9 or 10 tubules, 8 is the usual number.   In view of these
findings, the homology attempted by Pitelka must be considered doubtful.

Vesicles of various sizes are associated with the cytopharynx of *Bodo* (Pitelka,
1961), *Ichthyobodo(Costia)necator* (Joyon and Lom 1966, 1969) and *Cyathomonas
truncata* (Mignot, 1965; Schuster, 1968).   In the case of *Cyathomonas*, Mignot (1965)
believed that these vesicles arise from the Golgi apparatus and Schuster (1968) has
suggested that they contain digestive enzymes which are ultimately emptied into
the food vacuoles.   In *Bodo* however, profiles showing an undulatory cytopharyngeal

membrane strongly suggest that the vesicles arise by pinocytosis although in the absence of tracer experiments this can only be conjecture.

Although in most respects the flagella of *Bodo* closely resemble those described from other kinetoplastid flagellates (Pyne, 1960; Anderson and Ellis, 1965; Vickerman, 1969), they do possess two features, namely the striations of the posterior flagellum and the extracellular interflagellar connective, which are not shared by other members of this group. The significance of these structures is not known, but an extracellular connection between the 2 flagella may go some way to explaining synchrony of these organelles during movement. The paraxial rod of both flagella arises from the proximal basal plate but in the closely related trypanosomatids it only becomes recognizable a short way along the flagellum.

The mitochondrion was probably first visualized by Whitmore (1911) who described a fibril from *Bodo asiaticus* (= *B. caudatus*) which ran from the kinetoplast to the posterior end of the cell. Alexeieff (1912) observed this *'fibrille sidérophile'* in *B. caudatus* and *B. edax* but reported that it could only be seen in flagellates from young cultures. A detailed study of this structure was made by Hollande (1936, 1942). He noted that it was a constant feature of all species of *Bodo* but that there was a species difference in the extent to which it was developed. Because the *'côte'* or *'cordon sidérophile'*, as Hollande called it, was best developed in *B. saltans*, he paid more attention to this species and described in some detail the path taken by it through the cell. As noted by Pitelka (1961), it seems probable that Hollande was describing the mitochondrion since electron microscopy shows that this is the only organelle which follows an identical path through the cell.

The enclosure of the DNA-containing kinetoplast in a dilatation of the mitochondrial tube is a feature uniting *Bodo* with members of the Trypanosomatidae. However, they differ in the organization of the kinetoplast for whereas in the Trypanosomatidae the kinetoplast is disk shaped with its component fibrils arranged antero-posteriorly, in *Bodo* it is spherical with its fibrils forming a reticulum. Although in all kinetoplastid flagellates the basal bodies of the flagella lie very close to the surface of the kinetoplast capsule, no physical connection between the two has been found. Such a connection has been sought because in many trypanosomatids the two structures are linked in morphogenesis and appear connected after cell rupture (Simpson, 1968). The present study suggests that in the case of *Bodo caudatus* no direct connection is possible because the two structures are separated by a thick electron dense pad. The observations made by Simpson (1968) on *Leishmania tarentolae* led him to suggest that the kinetoplast capsule is attached to the basal body by an EDTA-sensitive cytoplasmic cement. There seems no reason why this explanation cannot be extended to other kinetoplastid flagellates.

Electron dense bodies similar to those found in the matrix of the mitochondrion have also been described from *Crithidia fasciculata* (Brooker, 1971) and in *Tetrahymena pyriformis* Levy and Elliott (1968) found that they become more numerous when the ciliates are starved. Although their significance is unknown, it is interesting that these bodies resemble the altered kinetoplast DNA of trypanosomatid flagellates which have been exposed to acriflavine (Kusel, Moore and Weber, 1967; Hill and Anderson, 1969). Although dyskinetoplastic *Bodo caudatus* was obtained

by Robertson (1929) using acriflavine, the electron miscroscopy of the kinetoplast after this treatment has not been studied.

The endocytoplasmic bacteria of *Bodo saltans* appear to be a constant feature of this species. Profiles showing constrictions across the equator of some bacteria support the assumption that they multiply to keep pace with division of the flagellate. Gram stained preparations of *B. saltans* provide little useful information since it is difficult to distinguish endocytoplasmic bacteria from food organisms. However, since the walls of Gram negative bacteria are very thin (Kellenberger and Ryter, 1958) compared with those of Gram positive bacteria (Glauert, 1962) the cytoplasmic bacteria of *Bodo* are judged to be Gram negative.

Bodies thought to be bacteria constantly occur in or on many species of protozoa as shown by the review of Kirby (1941). In most cases their identity and function is unknown. Since, in the case of *Bodo saltans*, there is no evidence to suggest that the flagellate benefits or is harmed by the association it is impossible at this stage to decide whether the bacteria are parasitic or symbiotic. However, in another kinetoplastid flagellate, namely *Crithidia oncopelti*, cytoplasmic bacteria or 'polar bodies' have been described by Newton and Horne (1957) which appear to provide the flagellate with lysine (Gill and Vogel, 1962).

Although there now appears to be general acceptance of the theory which holds that trypanosomes originated from the intestinal flagellates of insects (Hoare, 1948; Baker, 1963), the origins of the family still seem unclear. The presence in all trypanosomatid flagellates of a barren basal body in addition to that which produces the single motile flagellum (Rudzinska and Vickerman, 1969) may indicate origin from a biflagellate ancestor. Since at some stage this is likely to have been a free living flagellate, possible descent from *Bodo* or a *Bodo*-like organism is worth a brief consideration. Although the kinetoplast-mitochondrion is an obvious character uniting both groups of flagellates, it appears that an organelle comparable to the cytopharynx of *Bodo* has also been retained in a more or less modified form by many trypanosomatids (Brooker, 1971). In trypanosomatids, it is a deep (cytopharynx) or shallow (cytostome) invagination of the cell membrane associated, as in *Bodo*, with a number of microtubules. In both cases this organelle is endocytotic but whereas in *Bodo* it is primarily concerned with the ingestion of bacteria, in those trypanosomatids which have been examined it appears to be pinocytotic (Steinert and Novikoff, 1960; Preston, 1969; Brooker, 1971). Qualitative and quantitative differences in the nature of food ingested by *Bodo* and trypanosomatids may go some way to explain observed differences both in cell shape and the spatial arrangement of some organelles. Thus, the cytopharynx of *Bodo*, unlike that of trypanosomatids, is situated some distance from the flagellar pocket in order to facilitate prey capture and virtually the entire posterior half of the cell is devoted to the accommodation of food vacuoles. It is proposed therefore that the adoption of a parasitic mode of life by a *Bodo*-like flagellate and the subsequent abandonment of bacterophagic nutrition could have produced changes in body form which, together with other physiological adaptations led to the emergence of an ancestral trypanosomatid. Although such changes would have resulted in the retention of the cytopharynx, it

is assumed that the loss of one flagellum and development of the relatively sparse microtubular system occurred at some later stage.

ACKNOWLEDGEMENTS

KEY TO ABBREVIATIONS USED IN THE PLATES

ax	axoneme	G	Golgi apparatus
b	bacterium	ger	granular endoplasmic reticulum
bb	basal body	ifc	interflagellar connective
bc	buccal cavity	k	kinetoplast
cb	cytoplasmic bacterium	m	mitochondrion
cbl	circumbuccal lappets	mast	mastigonemes
cv	contractile vacuole	mt	microtubule
cyt	cytopharynx	n	nucleus
f	flagellum	pr	paraxial rod
fp	flagellar pocket	rt	rootlet
fv	food vacuole	ves	vesicle

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Fig. A.   Longitudinal section through the anterior end of *B. caudatus*.   The buccal cavity is seen in transverse section.   A bacterium in an early stage of ingestion occupies the greatly distended buccal cavity.   Note the dense pad (arrowheads) lying between the basal body of the flagellum and the kinetoplast capsule.   × 29,500.

Fig. B.   Longitudinal section through the basal plate region of two flagella showing the striated interflagellar connective.   × 96,000.

Fig. C.   Transverse section of two flagella at the level of the basal plates which are joined by the interflagellar connective.   × 54,000.

Fig. D.   Transverse section of the basal bodies of *B. saltans*.   They are connected by striated rootlets.   The rootlet passing from the basal body of the anterior flagellum to the vicinity of the cytopharynx can just be seen (arrow).   This cell is in the early stages of division and the daughter basal bodies are already forming.   A rootlet connects the basal body of the anterior flagellum with its daughter.   × 56,500.

Fig. E.   Transverse section of the posterior flagellum of *B. caudatus* showing the axoneme and the paraxial rod.   On one side, a flagellar striation is seen as a thickening of the flagellar membrane associated with sub-membrane material.   × 56,500.

Fig. F.   Posterior flagellum of *B. saltans* showing the parallel striations.   Metal shadowed. × 44,500.
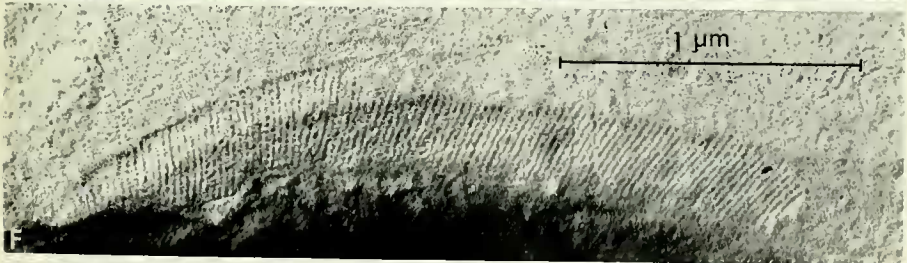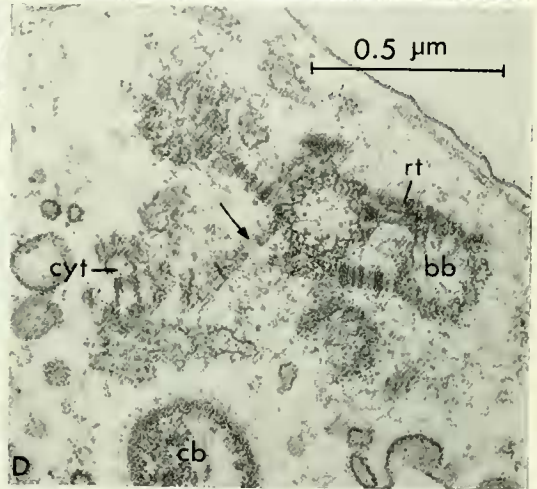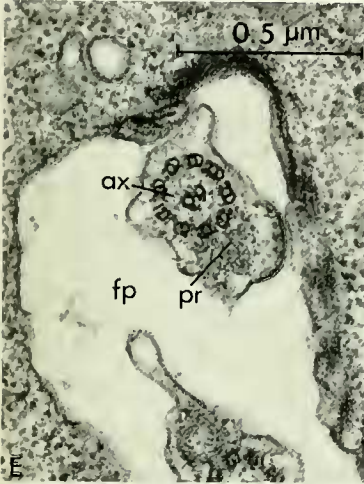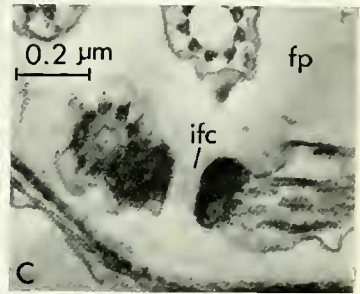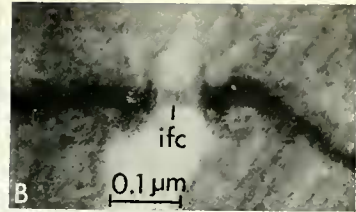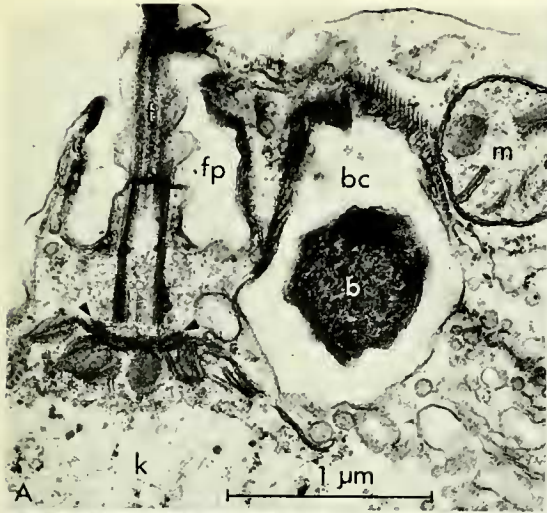
PLATE 2

Fig. A. Longitudinal section through the posterior flagellum of *B. caudatus* showing the striations of the flagellar membrane. · 56,500.

Fig. B. Transverse section through *B. saltans* showing the relationship of cytopharynx to the flagellar pocket. Small portions of the circumbuccal lappets are visible. One of the basal body rootlets appears clearly. Note the abundant vesicles lying on one side of the cytopharynx. · 29,500.

Fig. C. Longitudinal section of the buccal cavity and part of the cytopharynx of *B. saltans*. Note the pronounced cell coat (arrowheads) of the buccal cavity membrane, the cytoplasmic bacteria and position of the lappets. The anterior end of the flagellate is at the top of the micrograph · 30,000

Fig. D. Longitudinal section of *B. caudatus* showing the anteriorly directed buccal cavity. The kinetoplast and nucleus lie very close to each other in this species. The anterior end of the flagellate is to the right of the micrograph. × 22,000.
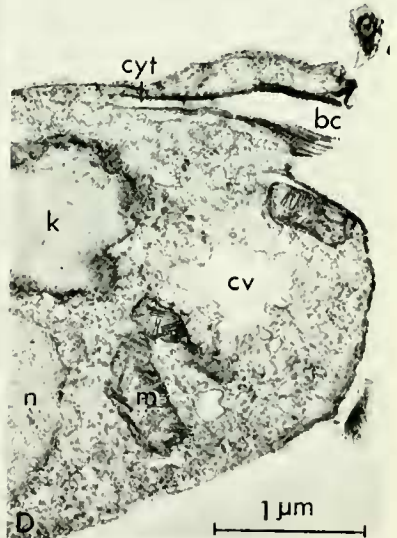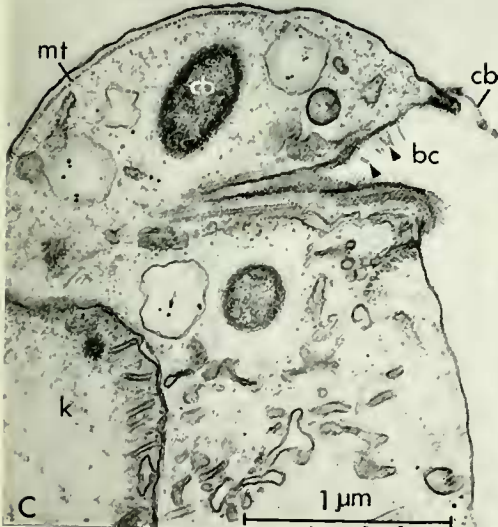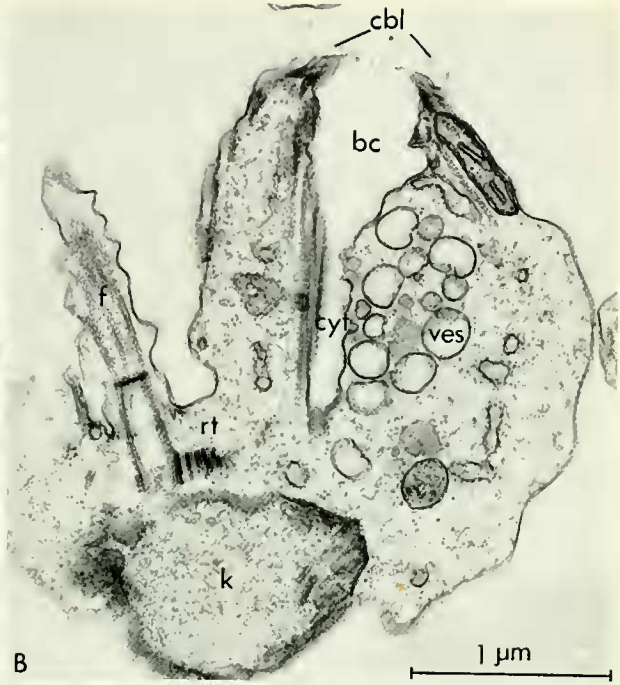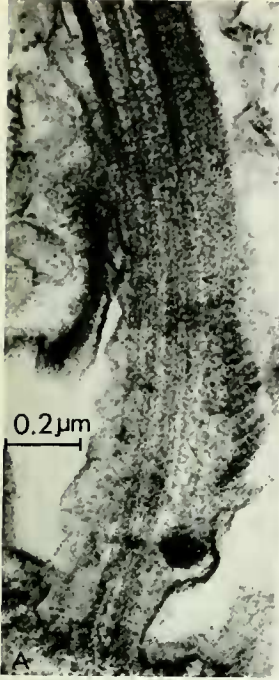
FIG. A. Negatively stained *B. saltans* showing the position of the circumbuccal lappets relative to the mastigonemes of the anterior flagellum. The position of the flagellum here corresponds to the bottom of the effective stroke. × 28,500

FIG. B. Section through the flagellar pocket of *B. saltans* demonstrating the pairing of microtubules shortly before they enter the buccal cavity. × 37,500.

FIG. C. Longitudinal section of *B. saltans* showing the undulatory path of the cytopharynx. The Golgi apparatus lies directly beneath this organelle. Note the numerous vesicles associated with the cytopharynx and the undulation of the cytopharyngeal membrane. A band of microtubules circumscribes the floor of the buccal cavity. × 45,000.

FIG. D. Transverse section of the cytopharynx of *B. saltans* showing eight microtubules of which three are paired. Note the vesicles lying beside it. × 66,000.

FIG. E. Transverse section of the cytopharynx of *B. saltans* showing that it is occasionally surrounded by nine microtubules. × 40,000
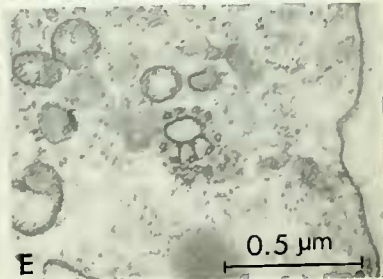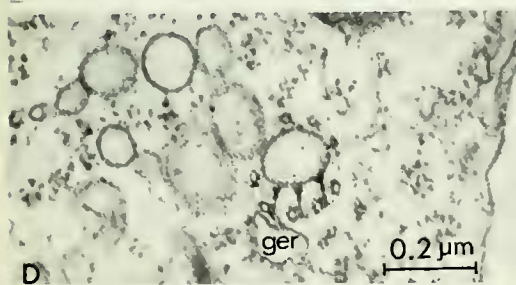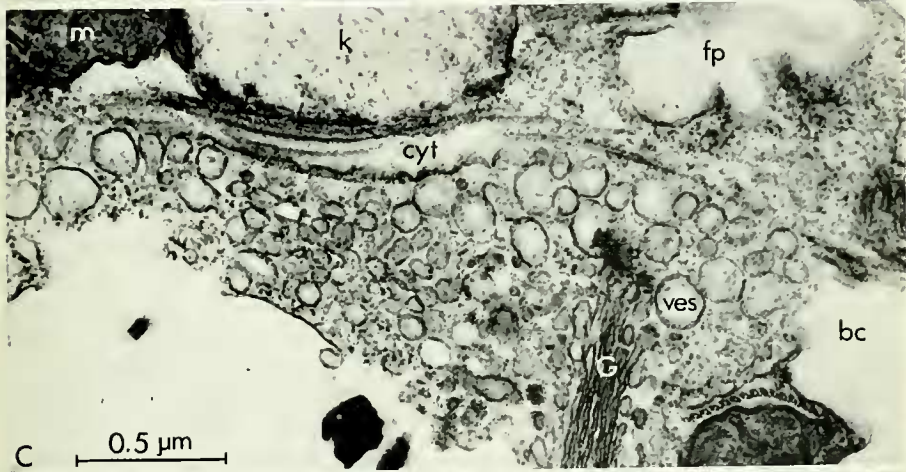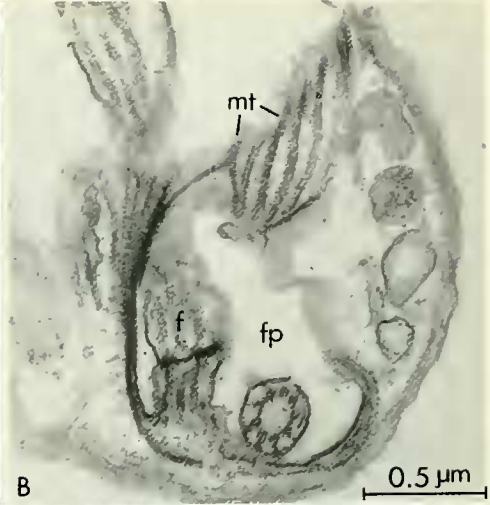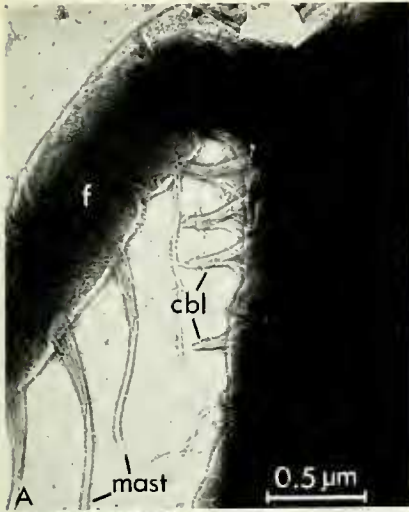
PLATE 3

FIG. A. Tangential section of the cytopharynx in *B. saltans*. Note the connectives joining adjacent tubule pairs. × 56,700.

FIG. B. Longitudinal section of *B. saltans* which passes through the flagellar pocket and cytopharynx transversely. The microtubule dipping below the surface of the cell is one of many which eventually pass under the buccal cavity as shown in Fig. C. Note the position of the cytopharynx and the vesicles lying one one side of it. × 30,000.

FIG. C. Transverse section of the buccal cavity of *B. saltans*. Note the tract of microtubules passing under the floor of this organelle. × 30,000.

FIG. D. Longitudinal section of *B. caudatus* which passes through the buccal cavity and flagellar pocket transversely. The contractile vacuole lies next to the flagellar pocket. Microtubules destined to surround the cytopharynx are arranged in a row in this section. Note the row of tubules associated with the opposite wall of the buccal cavity. × 40,000.

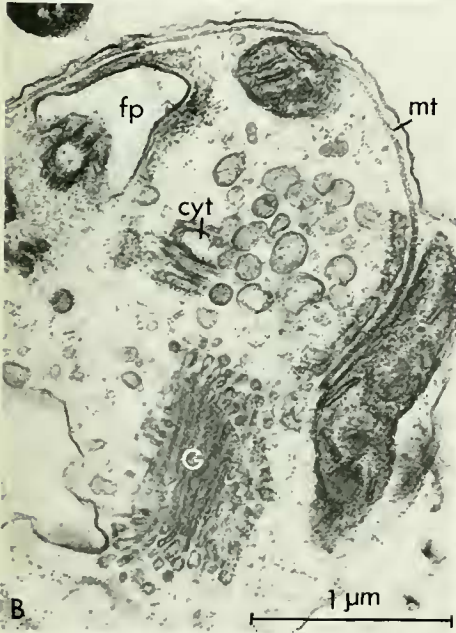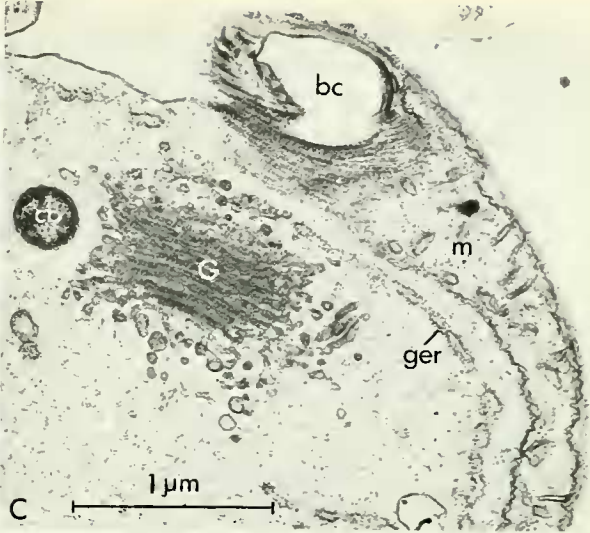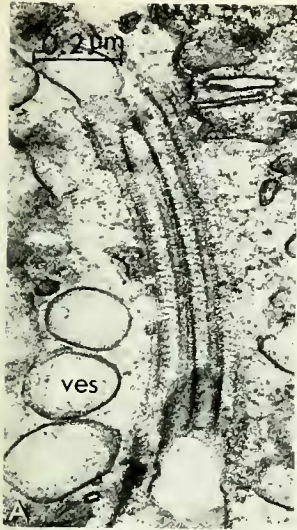*Bull. Br. Mus. nat. Hist.* (Zool.) 22, 3
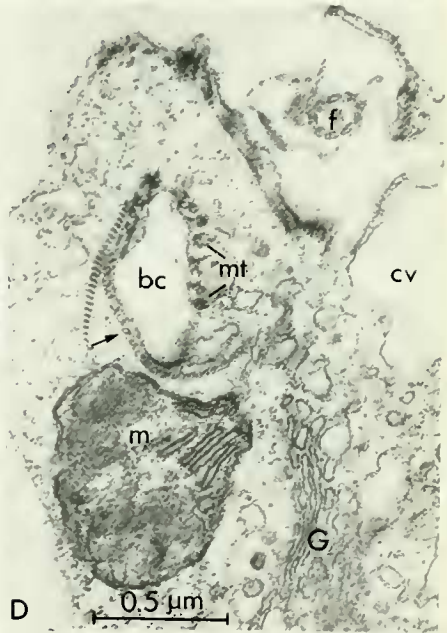
PLATE 4

PLATE 5

FIG. A   Transverse section of the buccal cavity posterior to that shown in Pl. 4, Fig. D. The microtubules associated with the buccal cavity (between arrows) progressively move away from it.   ⋅ 50,000.

FIG. B.   Transverse section through the beginning of the cytopharynx of *B. caudatus*.   The microtubules are arranging themselves around its walls.   Microtubules associated with the cytopharynx are the same set as shown in the previous figure (between arrows).   ⁄ 50,000.

FIG. C.   Section of the mitochondrion showing the electron dense bodies which are sometimes found in the matrix.   ⋅ 24,000.

FIG. D   Longitudinal section through *B. saltans* showing the mitochondrion passing to one side of the buccal cavity before travelling to the posterior end of the cell.   Other features include the nucleus, food vacuoles and Golgi apparatus.   ⁄ 15,500.
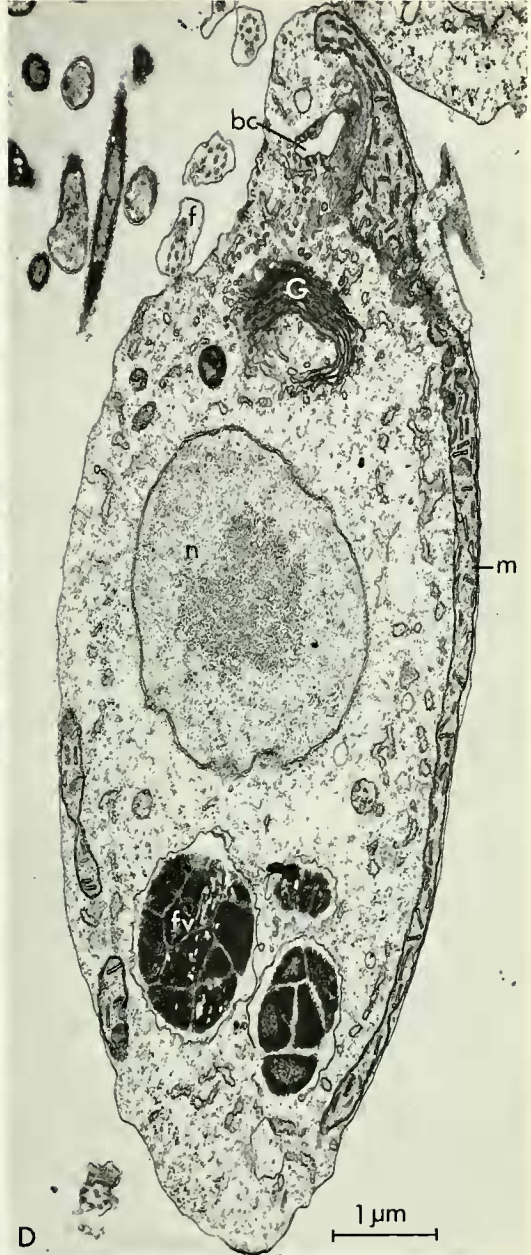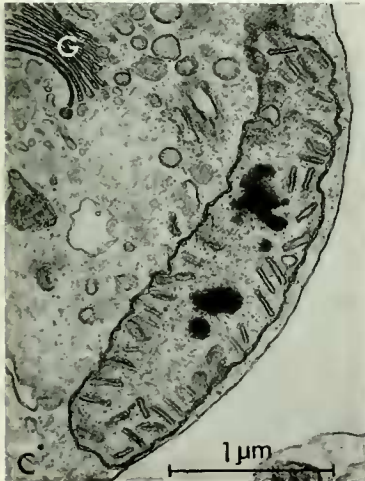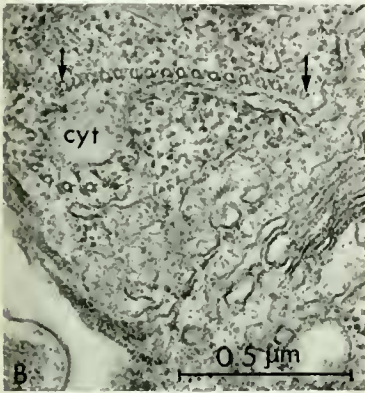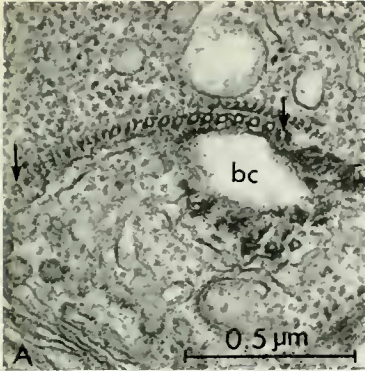
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PLATE 5

Fig A.   Section through the flat plane of the kinetoplast of *B. caudatus*.   Note that the kinetoplast is composed of a reticulum of fine filaments with electron dense nodes interspersed between them.   The matrix of the mitochondrion is visible at the periphery of the kinetoplast · 40,000.

Fig. B.   Contractile vacuole and spongiome of *B. saltans*.   · 24,000.

Fig. C.   Cytoplasmic bacterium of *B. saltans* in division.   ⁄ 46,000.

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PLATE 6